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December 21, 2004

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APPLICATION NUMBER: *60/519,140*
FILING DATE: *November 12, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/37813*

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
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PTO/SB/16 (05-03)
Approved for use through 4/30/2003. OMB 0651-0032
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. ER380175284US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Desuo	Wang	Columbia, SC
Walter J.	Sowell	Columbia, SC

Additional inventors are being named on the _____ separately numbered sheets attached hereto

TITLE OF THE INVENTION (500 characters max)

Compounds for Treating Cardiovascular Conditions and Asthma

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number _____ → Place Customer Number Bar Code Label here

OR *Type Customer Number here*

☑ Firm or Individual Name	University of South Carolina				
Address	Osborne Administration Building				
Address	Room 109				
City	Columbia	State	SC	ZIP	29208
Country	USA	Telephone	803-777-7854	Fax	803-777-9500

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 38 ☐ CD(s), Number

☐ Drawing(s) *Number of Sheets* ☐ Other (specify)

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

☐ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: _____

☑ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT ($) $80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☑ Yes, the name of the U.S. Government agency and the Government contract number are: National Institute of Health 5 K2200367-02

Respectfully submitted,

SIGNATURE Walter H. Parham

TYPED or PRINTED NAME Walter H. Parham

TELEPHONE 803-777-7854

Date 11/12/03

REGISTRATION NO. *(If appropriate)*

Docket Number: 341.00.PPA01r2

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SB/16 (05-03)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Docket Number	341.02-PPA

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Joseph W.	Kosh	Columbia, SC
Ting	Wang	Columbia, SC

Number 2 of 2

Provisional Patent Application

Deseuo Wang, J.W. Sowell, Joseph W. Kosh, and Ting Wang
University of South Carolina
USCRF Invnetion Disclosure #341

Compounds for treating cardiovascular conditions and asthma

We claim the invention having the embodiments described herein.

IDF No. (assigned by USCRF): 00341
Disclosure Date (date recv'd by USCRF): 05/20/02

INVENTION DISCLOSURE FORM

UNIVERSITY OF SOUTH CAROLINA RESEARCH FOUNDATION

In addition to their significant academic and scientific value, many of the inventions, creations, and/or discoveries made by employees and students of the University of South Carolina (hereinafter the "University") have potential commercial value. Therefore, University policy requires that employees and students disclose all such inventions, creations, and discoveries to the University of South Carolina Research Foundation (hereinafter "USCRF").

This Invention Disclosure Form has been designed to permit University inventors to provide timely and effective notification of their inventions to USCRF. Please fill out each of the sections and attachments as completely as possible.

This disclosure should be considered **confidential and proprietary**. Please note that early publication, dissemination, or public use of this invention, creation, or discovery may adversely affect the legal protection of your technology.

NOTE: You must sign the form in Section II, obtain department head and dean/director signatures in Section III, and obtain witness signatures (Section IV) for USCRF to begin to administer your invention or creation, which may include seeking adequate legal protection and exploring licensing and other commercialization opportunities.

SECTION I. Title of Invention and Primary Contact

1. *Non-enabling* title of work: Clinical use of SW0502 compounds to treat cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stroke and heart failure) and spasmodic asthma.

2. Name of contact inventor (who will be speaking and acting on behalf of the inventors listed on page 2):

Desuo Wang

SECTION II. Declaration of Ownership, Inventors, Royalty-sharing, and Assignment

For details on disclosures, categories and royalty sharing, see the Intellectual Property Management Office homepage http://ip.research.sc.edu or the faculty manual http://www.sc.edu/policies/facman/research.html..

1. Please check below to indicate Category of disclosure:

☐ Category 1 - No significant use of University funds, facilities, personnel or other University contribution.

☒ Category 2 - Invention/discovery made with University funding derived from non-University (e.g. - federal, industrial) sources.

☐ Category 3 - Invention/discovery made with University funding from University (e.g. - department, center, foundation) sources.

Note: As used above, University funding includes any funds that flow through the University, USCRF, or any other University-affiliated center or foundation, regardless of origin or funding agency.

2. If Category 2 or 3. I/we agree with the University's standard royalty sharing policy wherein the inventor(s) are entitled to receive 40% of the net licensing income received by the USCRF which is attributed to my/our invention.

☒ Yes

☐ No If no, please explain:

3. Please list below all individuals who will share in the inventors' share of licensing income, if any. Note that with regard to patents, the question of inventorship is a matter of law; failure to list every inventor who contributed to the conception or effective reduction to practice of the invention will invalidate any patent that may issue for this invention. Thus, while being named below entitles one to a share of the inventors' royalties. it does not necessarily ensure that each person listed will be named as an inventor on any patent(s) that may issue. If more than two co-inventors, please use Attachment 1.

As indicated by their signatures below, and subject to the University's policy on intellectual property, the following individuals do hereby:

A. assign their individual rights and ownership in this invention, creation, or discovery to the University of South Carolina Research Foundation, that the University of South Carolina Research Foundation may, in it's sole discretion, administer, protect, license, or otherwise use or exploit this invention for the benefit of the University, USCRF, and the individual inventors; and

B. agree that their portion of the inventors' share of net income resulting from any licensing, sale, or other commercialization of the invention is to be divided among themselves according to the percentages shown below; and

C. acknowledge that the abstract provided with this form in Attachment 2 represents a **non-enabling** description of the technology and may be released, published, or disseminated by the Intellectual Property Management Office in order to attract potential licensing candidates or to assess a potential financial return from this discovery; and

D. certify that all information provided in this disclosure is true and accurate to the best of their knowledge; and

E. agree that the individual whose name appears on page 1 of this form in Section I, no. 2, will be the contact point with the Intellectual Property Management Office on behalf of all those listed below. This contact inventor may or may not be the first inventor involved in this discovery; however, he or she should be one of the inventors, and should be listed below as such.

Please print or type:

1. Name: Desuo Wang — SSN: 591-86-1245

Assistant Professor (Title/position) — 700 Sumter St. (Mailing address)

Basic Pharmaceutical Sciences, College of Pharmacy (Dept/College)

Columbia, SC 29208 (City, State, Zip)

Inventor's share: 60 (%)

Desuo Wang (Signature)

05-06-02 (Date)

Office phone: 803-777-7101
Office fax: 803-777-8356
Email: wang@cop.sc.edu

2. Name: J. Walter Sowell, Sr. — SSN: 436-62-7314

Professor (Title/position) — 700 Sumter St. (Mailing address)

Basic Pharmaceutical Sciences, College of Pharmacy (Dept/College)

Columbia, SC 29208 (City, State, Zip)

Inventor's share: 25 (%)

J. Walter Sowell (Signature)

05/06/2002 (Date)

Office phone: 803-777-7916
Office fax: 803-777-8356
Email: sowell@cop.sc.edu

Note: If there are more than 2 inventors, please use Attachment 1.

SECTION III. Notifications

The following individuals must sign this Invention Disclosure Form before USCRF can formally begin to administer this invention.

Department Head

Joseph W Kosh
Signature

May 6, 2002
Date

Name (please print): Joseph W. Kosh

Department: Basic Pharmaceutical Sciences

College/Institute: College of Pharmacy

Dean/Director

Farid Sadik
Signature

5/6/02
Date

Name (please print): Farid Sadik

College/Institute: College of Pharmacy

SECTION IV. Witnesses.

Two witnesses (not co-inventors of the invention) must sign this form. They should be individuals who can attest to the authorship of the invention, are familiar with the history of the invention, and understand the principles involved in the invention. The Department Head and/or Dean may serve as witnesses if they meet the witness criteria.

1. Name of first witness: James M. Chapman
   Title/position: Associate Professor
   Telephone: 803-777-7926
   USC Department/College **OR** Basic Pharmaceutical Sciences
   Non-USC employee mailing address:
   City, State, and Zip: Columbia, SC 29208

James M. Chapman Jr.
Farid Sadik
Signature of Witness

5/7/02
5/6/02
Date

2. Name of second witness: Farid Sadik
   Title/position: Dean/Professor
   Telephone: 803-777-4151
   USC Department/College **OR** College of Pharmacy
   Non-USC employee mailing address:
   City, State, and Zip: Columbia, SC 29208

Please return this completed form to: Invention Disclosures
University of South Carolina Research Foundation
514 Byrnes Building
Columbia, SC 29208

If you have any questions, please call (803) 777-4031 or (803) 777-9515.

Abbreviated title: Clinical use of SW0502 Primary contact: Desuo Wang, Ph.D.

Attachment 1 Additional Inventors

1. Name: Joseph W. Kosh SSN: 462-60-4820

Professor
Title/position

Basic Pharmaceutical Sciences, College of Pharmacy
Dept/College

Inventor's share: 10 (%)

Joseph W Kosh
Signature

May 6, 2002
Date

700 Sumter St.
Mailing address

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Office phone: 803-777-3465
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2. Name: Ting Wang SSN: 657-10-7137

Graduate Assistant
Title/position

Basic Pharmaceutical Sciences, College of Pharmacy
Dept/College

Inventor's share: 5 (%)

Signature

05/10/02
Date

700 Sumter. St.
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Columbia, SC 29208
City, State, Zip

Office phone: 803-576-6300
Office fax: 803-777-8356
Email: ting@cop.sc.edu

Copy and attach this page as needed for additional inventors.

Abbreviated title: Clinical use of SW0520 Primary contact: Desuo Wang, Ph.D.

Attachment 2 Description of Invention

1. Please attach a *non-enabling* abstract of the work in *layman's terms*, including practical uses for the work. This abstract should be worded so that an expert in the field would *not* be able to reproduce the invention.

2. Conception date: February 1, 2002 and place: CLS 611, College of Pharmacy, USC

   Laboratory notebook or other documentation available? Yes ☒ No ____
   (If No, what proof of inventorship can you provide?)

3. Please attach a complete description of the invention/creation/discovery, including potential industrial applications. Feel free to attach papers, presentations, etc. that describe the technology.

   See attachment

4. Describe why the invention is better or more advantageous than present state-of-the-art technology. What benefits does it provide? What problems does it solve? (Attach papers/presentations/abstracts that address these questions.)
   The responses elicited by SW0502 compounds are faster and stronger than that induced by commonly used antihypertensive drugs like beta-adrenergic blockers or calcium channel blockers, but slower and weaker than that caused by nitroglycerin. The compound reduces heart rate and myocardium contraction markedly less than beta-adrenergic blockers and calcium channel blockers. These pharmacological properties craft SW0502 the saver and better choice in treatment of hypertensive condition, hypertention-related stroke and heart failure. The ability of relaxation of bronchial smooth muscles indicates that the compounds can be used to treat patients with spasmodic asthma, especially, in asthma patients who also have high blood pressure. The effectiveness when applied intravenously and orally ensures the feasibility of using the compounds to treat either hypertensive crises or sustained hypertensive conditions. In addition, the relatively low cost of the SW0502 compounds will make SW0502 a better choice in clinical practice.

5. List up to ten (10) key words that help describe or that relate to the work:
   2-diethylaminoacetamido-3-carbamyl-4-methylpyrroles, lidocaine analogs, local anesthetic, antiarrhythmic, vasodepressor, antihypertensive, angina pectoris, stroke, heart failure, spasmodic asthma.

6. Have products, apparatus, or compositions, etc. actually been made? Tested? Is there a prototype? How would you describe the present state of development?
   The products have been made and preliminary tests have been performed. There is a prototype of compounds we are working with.
   Currently we are 1) modifying the structure of the parent compound to identify the optimal chemical agent that has the maximal therapeutic benefits and causes minimum adverse events; 2) investigating the mechanism(s) that underlie the pharmaceutical effects of SW0502 compounds.

7. Is a company(ies) ready to license the invention? Yes ☐ No ☒
If yes, please list the company(ies) name(s), contact person(s), address(es), and phone number(s).

8. Please list all companies you believe are/may be/should be interested in your discovery. Please list individual contacts at each of these companies, if available, with addresses and phone numbers.
Companies potentially interested in our discovery:

CV Therapeutics
Eli Lilly and Company
Novartis
Merck & Co.
AstraZeneca
Chugai Biopharmaceuticals, Inc.
COR Therapeutics

Abbreviated title: Clinical use of SW0502 compounds Primary contact: Desuo Wang, Ph.D.

Attachment 3 Public Disclosures

1. Has the invention been described or discussed in any journal, abstract, paper, oral presentation, news story, thesis, dissertation or other medium? Yes ☐ No ☒

   If yes, date_____, medium (journal, conference, etc.):_____

   Name of journal, conference, etc.:_____

   Attach copy of abstract, presentation, thesis/ dissertation, etc.

2. Has there been any past public use, sale, or offer for sale of the invention? Yes ☐ No ☒

   If yes, date:_____, describe circumstances, including contact person(s), etc. below:

3. Planned publication or other disclosure? Yes ☒ No ☐

   If yes, date:May 1, 2004, medium (journal, conference, etc.):Journal and Conference

   Name of journal, conference, etc.:Circulation; Circulation Research; Scientific Conference of American Heart Association.

   Attach copy of abstract, presentation, thesis/ dissertation, etc.

4. Has the invention been disclosed to industry representatives? Yes ☐ No ☒

   If yes, date _____, describe circumstances, including contact person(s), etc. below:

5. Any other disclosures of the invention/creation? If so, please explain:
   none

Abbreviated title: Clinical use of SW0502 compounds Primary contact: Desuo Wang, Ph.D.

Attachment 4 Research Funding, Sponsorship, and Support Information

List *all* of the funding agencies, companies, organizations and sponsors of the research that led to this discovery or invention, *including those who supplied materials under a formal materials transfer agreement (MTA) prior to invention or reduction to practice*. If none, so state. If more space required, copy this page or attach additional pages as needed.

Name of sponsor (agency/foundation/company/etc.)	NIEHS/NIH
Sponsor's grant/project number	5 K2200367-02
USCRF, SPAR, or USC Foundation account number	11110-FA01
Principal investigator	Desuo Wang
Grant/contract/project title	Effects of Dioxins (TCDD) on Cardiac Ion Channels

Name of sponsor (agency/foundation/company/etc.)	
Sponsor's grant/project number	
USCRF, SPAR, or USC Foundation account number	
Principal investigator	
Grant/contract/project title	

Name of materials provider, if any	
USCRF/USC MTA number	
Related research agreement number, if any	
Related grant/contract/project title, if any	

What University support has this invention or discovery received?

Facilities: yes
Funding: no
Services: no
Other support (e.g., release time, paid technical assistance, etc): no

Are/were there any other sources of support for this invention or discovery? If so, please explain:
no

Clinical use of SW0502 compounds to treat cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stroke and heart failure) and spasmodic asthma

Desuo Wang, J. Walter Sowell, Joseph W. Kosh, Ting Wang

May 6, 2002

Abstract

We have discovered a group of chemical compounds (SW0502 family) that can markedly relax high potassium-induced contracture (static muscle shortening) of artery (rat and pig), vein (pig), and bronchial (rat) smooth muscles. In whole animal studies of SD rats, the leading compound reduces the blood pressure when it was administrated intravenously or *via* oral gavage at concentrations of 1-20 mg/kg body weight. The depressing effects on smooth muscle contracture and blood pressure are concentration-dependant and reversible upon removal of the compound. The compound-elicited responses are faster and stronger than that induced by commonly used antihypertensive drugs like β-adrenergic blockers or calcium channel blockers, but slower and weaker than that caused by nitroglycerin. The compound reduces heart rate and myocardium contraction markedly less than β-adrenergic blockers and calcium channel blockers. Based on these observations, we claim the sole right to use these compounds in treatment of cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stoke and heart failure) and spasmodic asthma.

Summary of Invention Disclosure

CONTACT:

Desuo Wang, Ph.D. & M.D. (Inventor)
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University of South Carolina
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Tel: 803-777-7101
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OR

Anthony M. Boccanfuso, Ph.D.
Managing Director
University of South Carolina Research Foundation
Room 501, Byrnes Building
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Tel: 803-777-4305
Fax: 803-777-4136
Email: amb@sc.edu

Compounds to treat cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stroke and heart failure) and spasmodic asthma

Abstract

A group of compounds has been discovered to be effective for the treatment of cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stoke and heart failure) and spasmodic asthma. Testing of the compounds has shown that they can markedly relax high potassium-induced contracture (static muscle shortening) of artery (tested in rat and pig), vein (tested in pig), and bronchial (tested in rat) smooth muscles. In whole animal studies of SD rats, the leading compound reduces the blood pressure when it was administered intravenously (1 mg/kg body weight) or *via* oral gavage (20 mg/kg body weight). The depressing effects on smooth muscle contracture and blood pressure are concentration-dependant and reversible upon removal of the compound. The compound-elicited responses are faster and stronger than that induced by commonly used antihypertensive drugs like β-adrenergic blockers or calcium channel blockers, but slower and weaker than that caused by nitroglycerin. The compound does not reduces heart rate and myocardium contraction as markedly as β-adrenergic blockers and calcium channel blockers.

Potential Areas of Use

The treatment of cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stoke and heart failure) and spasmodic asthma.

Main Advantages

Responses are faster and stronger than commonly used antihypertensive drugs.
Less adverse effects (less inhibition on heart rate and myocardium contraction).
Reduced therapy cost.

Development Status

Compound synthesis has been completed.
Preliminary data have been collected in both tissue and whole animal levels.
Experiments have been designed to investigate the underlying mechanism(s) of action.

Demonstration/Validation Status

In-vitro testing on artery, vein, and bronchial smooth muscles from rats and pigs.
In Langendorff-perfused isolated rat heart, testing on left ventricular developing pressure(a measurement of myocardial contractility) and coronary flow-rate.
In-vivo testing on carotid arterial blood pressure and electrocardiogram following intravenous bolus injection and oral gavage administration.

Future Development Plan

Toxicity of the compound will be studied.
Pharmacokinetics (uptake, distribution, binding, metabolism, and elimination) will be studied.
Experiments will be implemented to investigate the underlying mechanism(s) of action.
Clinical trail bases have been established.

Introduction

Hypertension is the most common cardiovascular disease and the management of hypertension is the leading indication for both visits to physicians and the use of prescription drugs in the United States. The elevated blood pressure is closely associated with high morbidity, disability, and mortality from coronary heart disease and strokes. Although antihypertensive therapy can effectively prevent the hemorrhagic strokes, cardiac failure, and renal insufficiency due to high blood pressure, epidemiological studies demonstrate that only 27 percent of hypertensives had their blood pressure well controlled.[1-4] These facts lead the discovery of new antihypertensive agents remains one of the major focuses of R&D of cardiovascular research.

The clinical treatment of hypertension is to prevent the cardiovascular complications that are known to accompany the high blood pressure.[5] Currently, five major classes of drugs are used to lower blood pressure, which include diuretics, adrenergic inhibitors, Ca^{2+}-channel blockers, renin-angiotensin inhibitors, and vasodilators.[6] The later, together with Ca^{2+} antagonists and angiotensin inhibitors, is becoming wider and earlier choice as first or second line of drugs to control blood pressure.[5]

We have discovered a group of chemical compounds that potently relax vascular and bronchial smooth muscles contractured by high potassium in vitro and lowered the blood pressure in vivo. We, therefore, claim that these compounds have great potential to be developed into new therapeutic agents to treat hypertension and hypertension-related heart diseases and stroke as well as spasmodic asthma.

Chemistry of the Compounds: **Omitted.**

Biological Evaluation

I. Smooth muscle relaxation

A. In vitro vascular smooth muscle responses:

The prototype of the compounds was tested for activity to relax vascular smooth muscles using abdominal aorta and vein preparations from Sprague-Dawley rats and Sus-Scrosa pigs. The rat vessels were obtained immediately after the animal was sacrificed for another research project (Animal protocol # 1091, approved by IACUC of University of South Carolina) and the porcine tissues were obtained within 30 min after the pig was sacrificed at the end of an Advanced Trauma Life Support Course (a training course organized by the College of Medicine and Animal Facility of USC). The vessels were cleaned of adherent fat and connective tissue and then cut into 3~5-mm ring segments. The segments were cut longitudinally into arterial strips without removal of the endothelium.

The strips were tied to the extremities by two silk threads and vertically mounted in a water-jacketed tissue bath, maintained at 37 °C, between moveable and fixed ends of a stainless steel wire with the moveable end attached to a TRI 201 isometric transducer (LSi LETICA Scientific Instruments) that was coupled to a bridge amplifier (PowerLab, ADIstruments) for recording isometric force responses. The data were digitized through a 16 SP interface and acquired on-line with Chart 4.1.2 software (PowerLab, ADIstruments) and stored to a personal computer.

The bath was filled with 30 ml of oxygenated (95%.O2 and 5% CO2) Kreb's solution containing (in mM): 135 NaCl, 15 $NaHCO_3$, 5.4 KCl, 1.2NaH_2PO_4, 1.2 $MgSO_4$, 1.8 $CaCl_2$, and 10 glucose. The strips were equilibrated for at least 30 min before approximately 0.3 gram (for rat tissues) or 1 gram (for pig tissues) of passive tension was placed on them. Thereafter, contracture (static

muscle shortening) was produced with potassium chloride by substituting 64.6 mM KCl for the same amount of NaCl in the Kreb solution (i.e., final concentration of K^+=70 mM). When the potassium-induced contracture reached a stable plateau level, various concentrations of the testing compound were added to the bath solution to record the concentration-response curve. Each concentration of the compound was allowed to elicit its maximal relaxation. In most of the experiments, the higher concentration was reached by accumulatively adding the agent to the bath solution. The relaxation response produced by the compound was normalized to the response produced by 10 μM nitroglycerin added to the bath solution at the end of the experiment. Figure 1 shows the typical vascular relaxation effects produced by the prototype of the compounds on rat arterial (panel *a*) and porcine vein (panel *b*) strips.

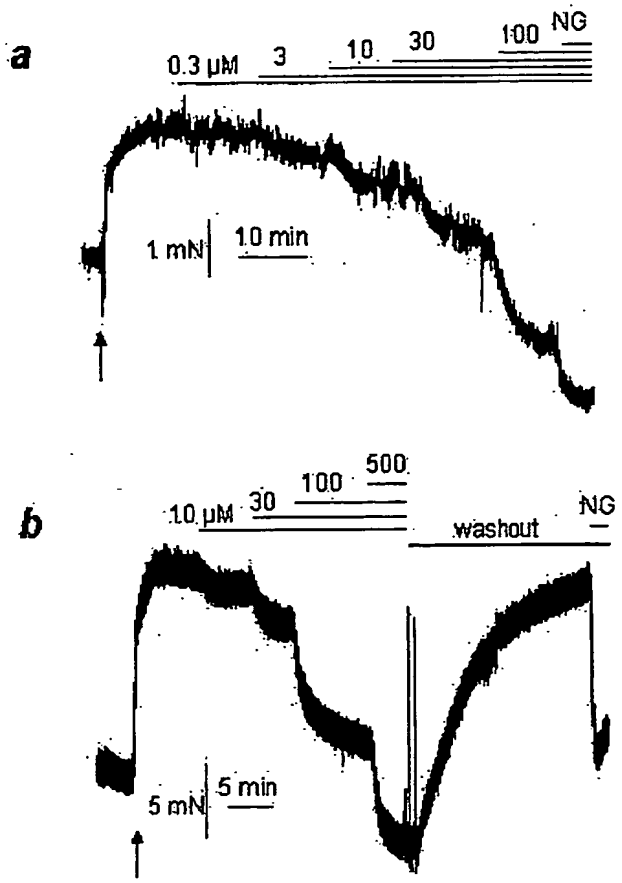

Figure 1. Prototype compound-produced relaxation of vascular smooth muscles. Panel a. rat abdominal arterial strip was statically shortened by 70 mM KCl indicated by the arrow. The test compound was added accumulatively at the concentration indicated by the numbers. NG=10 μM nitroglycerin. Panel b. similar experiment as in Panel a, except that porcine vein strip was used.

B. In vitro bronchial smooth muscle responses:

The prototype of the compounds was also tested for activity to relax bronchial smooth muscles using the main bronchi from Sprague-Dawley rats. The rat bronchial tissues were obtained immediately after the rat was sacrificed for another research project (Animal protocol # 1091, approved by IACUC of USC). Both the right and left main bronchi were cleaned of adherent fat and connective tissue and trimmed into ~3 mm ring segments, which were cut longitudinally at the

circumference of the hyaline cartilage without removal of the epithelium. The strips, with the membranous wall (a fibrous membrane containing smooth muscular fibers) in the middle, were tied to the extremities (cartilage+annular ligament) by two silk threads and vertically mounted in a water-jacketed 37 °C tissue bath. The experimental protocol and data acquisition were same as that used for vascular smooth muscles (see above). Figure 2 shows the typical bronchial responses produced by the compound.

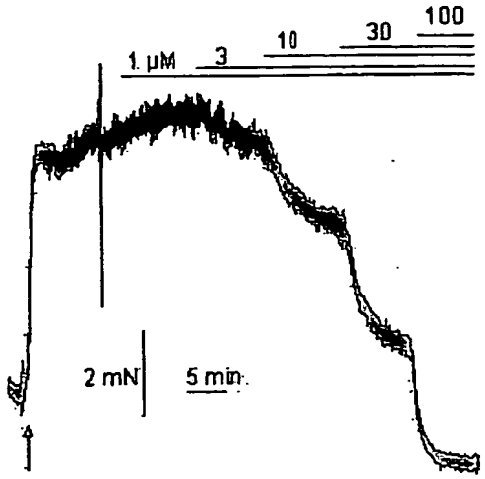

Figure 2. The prototype compound-produced relaxation of rat bronchial strip that was statically shortened by 70 mM KCl as indicated by the arrow. The bars and numbers indicate the application time and concentrations of the agent, respectively.

II. In vivo blood pressure reduction effects

A. Intravenous bolus injection of the compound on blood pressure:

Female Sprague-Dawley rats weighing 200-225 grams were anesthetized with pentobarbital (30 mg/kg body weight, i.p.). The animal was positioned supinely on a heat plate that was controlled by an automatic temperature controller system (TC-324B, Warner Instrument Corp.) to maintain its body temperature constant (36+-0.5C) during the experiment. The trachea, right carotid artery, and left femoral vein were exposed. The trachea was cannulated and the animal was breathed with air through a respirator at a rate of 65-70/min and an inspiration pressure of ~10 cm H_2O. PE catheters were implanted into the carotid artery and femoral vein, respectively. The animal was heparinized by i.v. injection of ~300 units of heparin dissolved in 0.3 ml of 0.9% saline. Arterial pressure was recorded from the carotid artery catheter connected to a physiological pressure transducer (SP 844, Capto company, Norway) that was coupled to a bridge amplifier (PowerLab, ADIstruments). The data were digitized through a 16 SP interface and acquired on-line with Chart 4.1.2 software (PowerLab, ADIstruments) and stored to a personal computer.

After surgery, the animal was allowed to equilibrate for ~ 30 minutes before administration of the test compounds. The experiments in which the control carotid pulse pressure (difference between the systolic and diastolic pressure) was less than 30 mmHg were discarded without testing the drug's effects. Various doses (0.1-4 mg/kg.bw) of the test agents dissolved in 0.9% saline were administered as boluses through the femoral vein cannula in volumes ranging from 0.25-0.3 ml. Same volume of saline was used to flush the injection line following each administration of drug. Each dose of the compound was allowed to elicit its maximal effects. The changes in systolic, diastolic, and mean

pressure (calculated as diastolic pressure+1/3 pulse pressure) caused by the test agents were compared to that measured at the end of the 30 min equilibration. During the experimental period, ECG was simultaneously recorded with a Bio Amplifier (PowerLab, ADIstruments) through two electrodes placed on the right and left forearms to monitor the possible effect of the test agents on heart rate. Figure 3 shows the representative responses of blood pressure and heart rate after bolus injection of the compound (note: the data shown in figure 3 were from the same rat. The lower mean blood pressure (MBP) and heart rate at the beginning of the recordings in Panel C were due to application of verapemil and nitroglycerin before lidocaine injection).

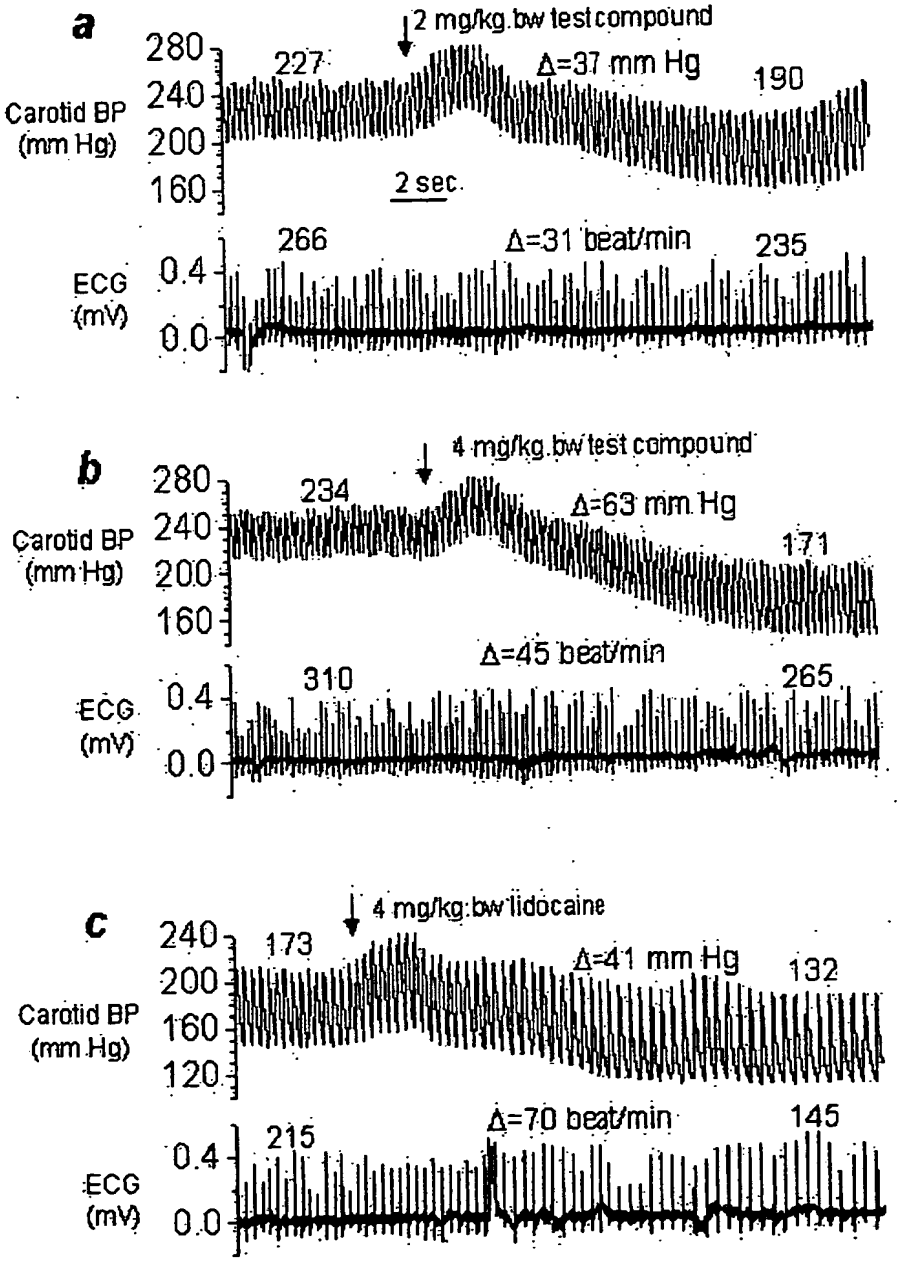

Figure 3. Effects of the prototype compound on rat carotid blood pressure and heart rate when administered intravenously (bolus injection, Panel a and b). Lidocaine's effects was included for comparison (Panel c). [The experiments were performed in the same rat. Lidocaine's effects were tested after administration of verapemil and nitroglycerin.]

B. Oral administration of the compound II on blood pressure:

The prototype of the compounds was administered to two female rats via oral gavage at a dose of 25 and 80 mg/kg.bw, respectively. The agent was dissolved in 0.9% saline in a volume of 0.5 ml. The same volume of saline alone was administered to two control animals. After 15 minutes, the animal was anesthetized with pentobarbital (30 mg/kg.bw, i.p.) and same surgery was performed except that the femoral vein was not exposed and there was no i.v. administration of the test agent. The carotid blood pressure and heart rate were monitored and recorded for 60 minutes using the same method described above. The initial recording was made within 45 to 65 minutes after performing oral gavage. The results are reported in the following Table (the MBP and HR were measured within 30 min after the surgery).

Oral gavage compound on rat blood pressure and heart rate

	Saline	Compound	
		25 mg/kg.bw	80 mg/kg.bw
MBP (mm Hg)	186	156	126
HR (beat/min)	364	384	300

MBP: mean blood pressure: HR: heart rate

Reference

1. The sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. Arch Intern Med 1997; 157:2413
2. Andersson OK, et al. Survival in treated hypertension: Follow up study after two decades. BJM 1998; 317:167-171
3. Woodwell DA: National Ambulatory Medical Care Survey: 1997 Summary. Advance Data from Vital and Health Statistics. 1999; National Center for Health Statistics. Hyattsville, MD; No. 305
4. MacMahon S, et al. Blood pressure and coronary heart disease: Part 1, prolonged differences in blood pressure: Prospective observational studies corrected for the regression dilution bias. Lancet 1990; 335:765
5. Braunwald E, Zipes DP, Libby P. Heart Disease. A textbook of cardiovascular medicine. (6th ed) W.B. Saunders Company. 2001; Vol 1, pp 972-995
6. Oates JA and Brown NJ. Antihypertensive agents and the drug therapy of hypertension. In Goodman & Gilman's The Pharmacological Basis of Therapeutics (10th Ed). Publisher: McGraw Hill; 2001; Chapter 33, pp 871-900

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[CONFIDENTIAL]

Summary of Invention Disclosure

May 17, 2002

**DEUO WANG*, J. WALTER SOWELL,
JOSEPH W. KOSH, TING WANG**

***Contact inventor**

Introduction

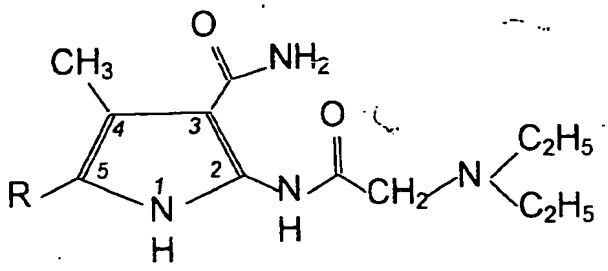
Hypertension is the most common cardiovascular disease and the management of hypertension is the leading indication for both visits to physicians and the use of prescription drugs in the United States. The elevated blood pressure is closely associated with high morbidity, disability, and mortality from coronary heart disease and strokes. Although antihypertensive therapy can effectively prevent the hemorrhagic strokes, cardiac failure, and renal insufficiency due to high blood pressure, epidemiological studies demonstrate that only 27 percent of hypertensives had their blood pressure well controlled.[1-4] These facts lead the discovery of new antihypertensive agents remains one of the major focuses of R&D of cardiovascular research.

The clinical treatment of hypertension is to prevent the cardiovascular complications that are known to accompany the high blood pressure.[5] Currently, five major classes of drugs are used to lower blood pressure, which include diuretics, adrenergic inhibitors, Ca^{2+}-channel blockers, renin-angiotensin inhibitors, and vasodilators.[6] The later, together with Ca^{2+} antagonists and angiotensin inhibitors, is becoming wider and earlier choice as first or second line of drugs to control blood pressure.[5]

We have discovered a group of compounds that potently relax vascular and bronchial smooth muscles, therefore, have great potential to be developed into new therapeutic agents to treat hypertension and hypertension-related heart diseases and stroke as well as spasmodic asthma.

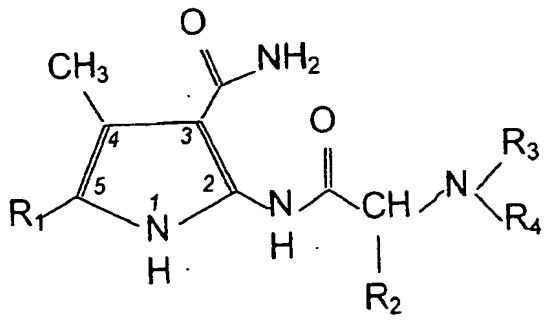
The prototype of the compounds was synthesized two decades ago,[7] when searching for better local anesthetic and antiarrhythemic agents. One of the original compounds, [2-(diethylamino) acetamido]-3-carbamyl-4-methyl-5-benzylpyrrole (Structure I) was reported to decrease blood pressure.[8-9] The therapeutic use of the benzyl compound was ruled out due to its low water solubility and its propensity to precipitate in blood.

With the availability of state-of-the art techniques and instruments for studying the mechanism of action of cardiovascular agents, we have recently evaluated the effects of [2-(diethylamino) acetamido]-3-carbamyl-4-methyl-5-methylthioethylpyrrole (Structure II) on smooth muscle contraction and on blood pressure. We discovered that this soluble compound markedly relaxed high potassium-caused smooth muscle contracture *in vitro* and lowered the blood pressure *in vivo*. By modifying the chemical structure, we defined a new group of chemical compounds that, like the prototype compound II, could have immense therapeutic value in treatment of cardiovascular disorders.



Compound	R
I	$-CH_2-C_6H_5$
II	$-CH_2CH_2SCH_3$

The general structure for our invention disclosure is given below. The R groups are inclusively defined, respectively.



where,

R_1=CH_3, CH_2CH_3, $CONH_2$, CH_2SCH_3, $CH_2SCH_2CH_3$, $CH_2CH_2SCH_3$, $CH_2CH_2SCH_2CH_3$, CH_2NCH_3, $CH_2NCH_2CH_3$, or the R group of any naturally occurring α-amino acid;

R_2=H, CH_3, CH_2CH_3, CH_2SCH_3, $CH_2SCH_2CH_3$; $CH_2CH_2SCH_3$, or $CH_2CH_2SCH_2CH_3$;

R_3=CH_3, C_2H_5, ηC_3H_7, ιC_3H_7, or ηC_4H_9;

R_4= CH_3, C_2H_5, ηC_3H_7, ιC_3H_7, or ηC_4H_9.

Biological Evaluation

I. Smooth muscle relaxation

A. In vitro vascular smooth muscle responses:

The prototype of the compounds (compound II) was tested for activity to relax vascular smooth muscles using abdominal aorta and vein preparations from Sprague-Dawley rats and Sus-Scrosa pigs. The rat vessels were obtained immediately after the animal was sacrificed for another research project (Animal protocol # 1091, approved by IACUC of USC) and the porcine tissues were obtained within 30 min after the pig was sacrificed at the end of an Advanced Trauma Life Support Course (a training course organized by the College of Medicine and Animal Facility of USC). The vessels were cleaned of adherent fat and connective tissue and then cut into 3~5-mm ring segments. The segments were cut longitudinally into arterial strips without removal of the endothelium.

The strips were tied to the extremities by two silk threads and vertically mounted in a water-jacketed tissue bath, maintained at 37 °C, between moveable and fixed ends of a stainless steel wire with the moveable end attached to a TRI 201 isometric transducer (LSi LETICA Scientific Instruments) that was coupled to a bridge amplifier (PowerLab, ADIstruments) for recording isometric force responses. The data were digitized through a 16 SP interface and acquired on-line with Chart 4.0 software (PowerLab, ADIstruments) and stored to a personal computer.

The bath was filled with 30 ml of oxygenated (95% O2 and 5% CO2) Kreb's solution containing (in mM): 135 NaCl, 15 $NaHCO_3$, 5.4 KCl, 1.2NaH_2PO_4, 1.2 $MgSO_4$, 1.8 $CaCl_2$, and 10 glucose. The strips were equilibrated for at least 30 min before approximately 0.3 gram (for rat tissues) or 1 gram (for pig tissues) of passive tension was placed on them. Thereafter, contracture (static muscle shortening) was produced with potassium chloride by substituting 64.6 mM KCl for the same

amount of NaCl in the Kreb solution (i.e., final concentration of K^+=70 mM). When the potassium-induced contracture reached a stable plateau level, various concentrations of the testing compound were added to the bath solution to record the concentration-response curve. Each concentration of the compound was allowed to elicit its maximal relaxation. In most of the experiments, the higher concentration was reached by accumulatively adding the agent to the bath solution. The relaxation response produced by the compound was normalized to the response produced by 10 μM nitroglycerin added to the bath solution at the end of the experiment. Figure 1 shows the typical vascular relaxation effects produced by the prototype of the compounds on porcine arterial (panel ***a***) and vein (panel ***b***) strips. Similar results were observed in rat tissues (not shown).

B. In vitro bronchial smooth muscle responses:

The compound II was also tested for activity to relax bronchial smooth muscles using the main bronchia from Sprague-Dawley rats. The rat bronchial tissues were obtained immediately after the rat was sacrificed for another research project (Animal protocol # 1091, approved by IACUC of USC). Both the right and left main bronchi were cleaned of adherent fat and connective tissue and trimmed into ~3 mm ring segments, which were cut longitudinally at the circumference of the hyaline cartilage without removal of the endothelium. The strips, with the membranous wall (a fibrous membrane containing smooth muscular fibers) in the middle, were tied to the extremities (cartilage+annular ligament) by two silk threads and vertically mounted in a water-jacketed 37 °C tissue bath. The experimental protocol and data acquisition were same as that used for rat vascular smooth muscles (see above). Figure 2 shows the typical bronchial relaxation responses produced by the prototype of the compounds.

II. In vivo blood pressure reduction effects

A. Intravenous bolus injection of the compound II on blood pressure:

Female Sprague-Dawley rats weighing 200-225 grams were anesthetized with pentobarbital (30 mg/kg body weight, i.p.). The animal was positioned supinely on a heat plate that was controlled by an automatic temperature controller system (TC-324B, Warner Instrument Corp.) to maintain its body temperature constant (36+-0.5C) during the experiment. The trachea, right carotid artery, and left femoral vein were exposed. The trachea was cannulated and the animal was breathed with air through a respirator at a rate of 65-70/min and an inspiration pressure of ~10 cm H_2O. PE catheters were implanted into the carotid artery and femoral vein, respectively. The animal was heparinized by i.v. injection of ~300 units of heparin dissolved in 0.3 ml of 0.9% saline. Arterial pressure was recorded from the carotid artery catheter connected to a physiological pressure transducer (SP 844, Capto company, Norway) that was coupled to a bridge amplifier (PowerLab, ADIstruments). The data were digitized through a 16 SP interface and acquired on-line with Chart 4.0 software (PowerLab, ADIstruments) and stored to a personal computer.

After surgery, the animal was allowed to equilibrate for ~ 30 minutes before administration of the test compounds. The experiments in which the control carotid pulse pressure (difference between the systolic and diastolic pressure) was less than 30 mmHg were discarded without testing the drug's effects. Various doses (0.1-4 mg/kg.bw) of the test agents dissolved in 0.9% saline were administered as boluses through the femoral vein cannula in volumes ranging from 0.25-0.3 ml. Same volume of saline was used to flush the injection line following each administration of drug. Each dose of the compound was allowed to elicit its maximal effects. The changes in systolic, diastolic, and mean pressure (calculated as diastolic pressure+1/3 pulse pressure) caused by the test agents were compared to that measured at the end of the 30 min equilibration. During the experimental period, ECG was simultaneously recorded with a Bio Amplifier (PowerLab, ADIstruments) through two electrodes placed on the right and left forearms to monitor the possible effect of the test agents on heart rate. Figure 3 shows the representative responses of blood pressure and heart rate after bolus injection of the

compound II (note: the data shown in figure 3 were from the same rat. The lower mean blood pressure (MBP) and heart rate at the beginning of recordings in Panel C were due to application of verapemil and nitroglycerin before lidocaine injection).

B. Oral administration of the compound II on blood pressure:

The compound II was administered to two female rats via oral gavage at a dose of 26 and 80 mg/kg.bw, respectively. The agent was dissolved in 0.9% saline in a volume of 0.5 ml. Same volume of saline alone was administered to two control animals. After 15 minutes, the animal was anesthetized with pentobarbital (30 mg/kg.bw, i.p.) and same surgery was performed except that the femoral vein was not exposed and there was no i.v. administration of the test agent. The carotid blood pressure and heart rate were monitored and recorded for 60 minutes using the same method described above. The initial recording was made within 45 to 65 minutes after performing oral gavage. The results are reported in the following Table (the MBP and HR were measured within 30 min after the surgery).

Oral Gavage Compound II on Blood Pressure and Heart Rate

	Saline	Compound II	
		26 mg/kg.bw	80 mg/kg.bw
MBP (mm Hg)	186	156	126
HR (Beet/min)	364	384	300

Also embraced within this invention is a class of pharmaceutical agents including one or more compounds of the formula structure in association with one or more non-toxic, pharmaceutically acceptable carrier materials such as diluents and adjuvants or other active/inactive ingredients. The invention covers all suitable and therapeutically effective administration routes, such as administrated intravascularly, intraperitoneally, subcutaneously, intramuscularly, or topically.

Reference

1. The sixth report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure. Arch Intern Med 1997; 157:2413
2. Andersson OK, et al. Survival in treated hypertension: Follow up study after two decades. BJM 1998; 317:167-171
3. Woodwell DA: National Ambulatory Medical Care Survey: 1997 Summary. Advance Data from Vital and Health Statistics. 1999; National Center for Health Statistics. Hyattsville, MD; No. 305
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6. Oates JA and Brown NJ. Antihypertensive agents and the drug therapy of hypertension. In Goodman & Gilman's The Pharmacological Basis of Therapeutics (10th Ed). Publisher: McGraw Hill; 2001; Chapter 33, pp 871-900
7. Johnson RW, et al. Synthesis of substituted 2-aminopyrrole analogs of lidocaine II. J. Pharmaceut. Sci. 1979; 68:955-958

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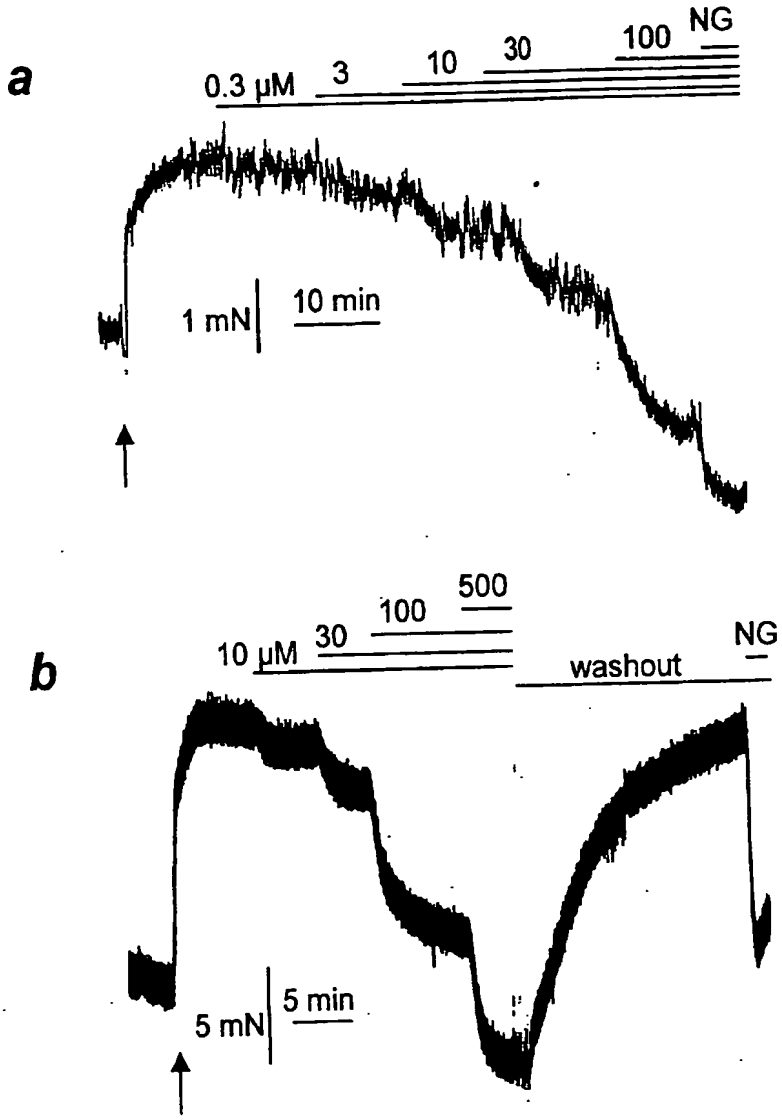

Figure 1. Prototype compound-produced relaxation of vascular smooth muscle. Panel a. rat abdominal arterial strip was statically shortened by 70 mM KCl indicated by the arrow. The test compound was added accumulatively at the concentration as indicated by the numbers. NG=10 μM nitroglycerin. Panel b. similar experiment as in Panel a, except that porcine vein strip was used.

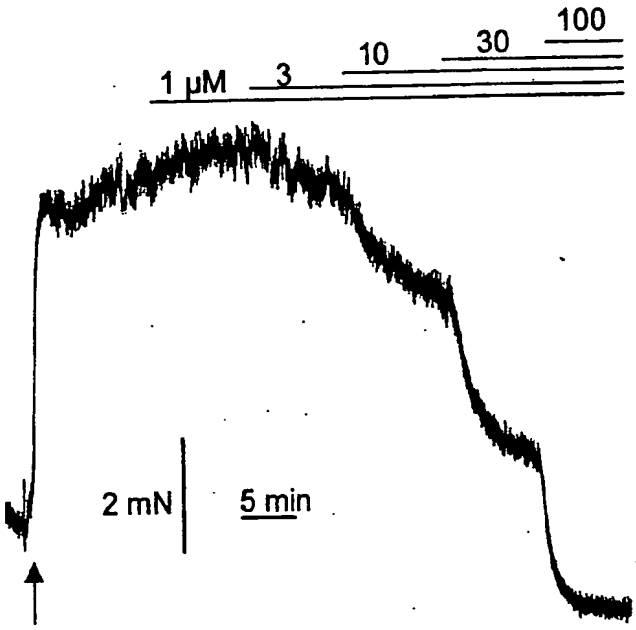

Figure 2. The prototype compound-produced relaxation of rat bronchial strip that was statically shortened by 70 mM KCl as indicated by the arrow. The bars and numbers indicate the application time and concentrations of the agent, respectively.

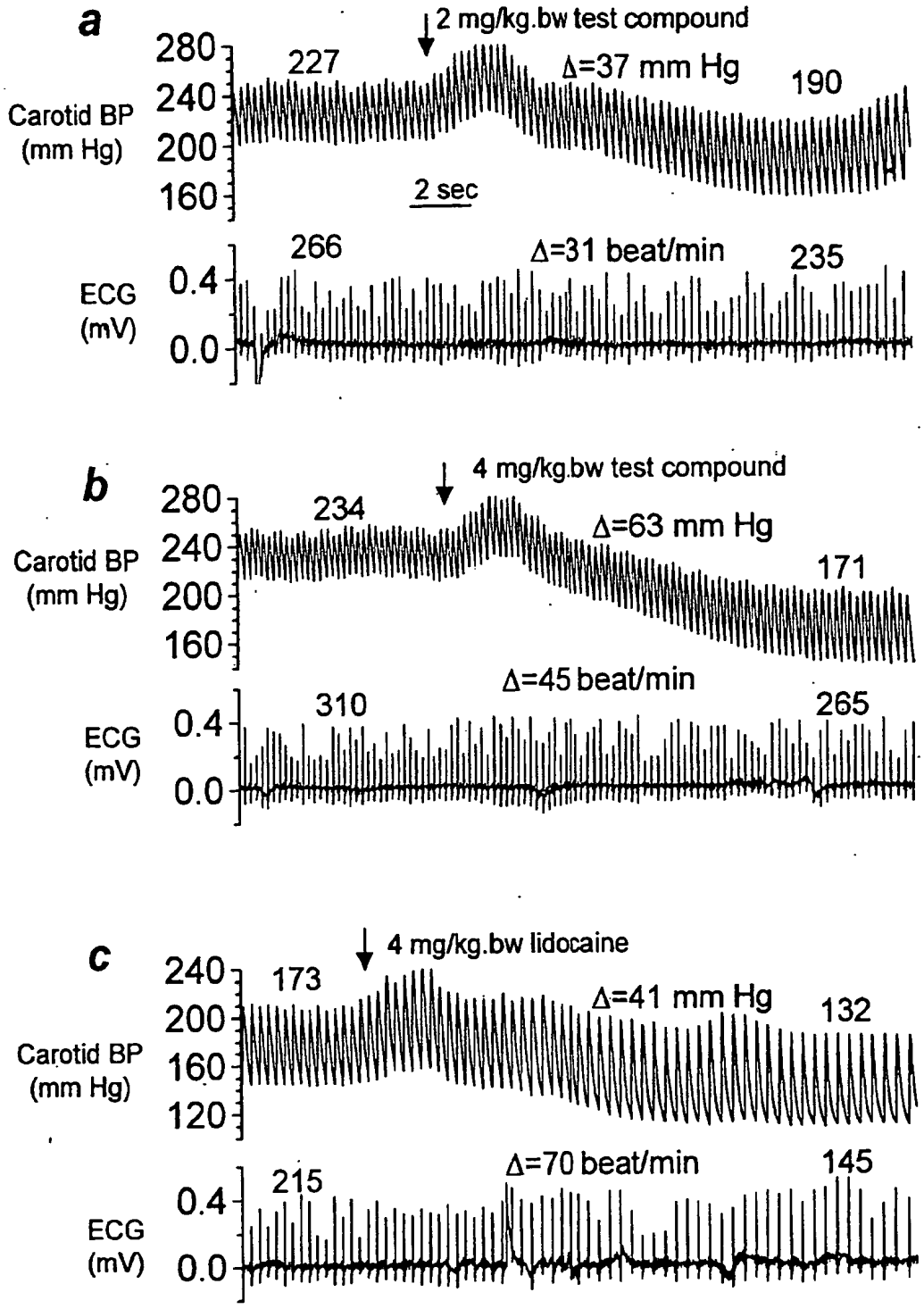

Figure 3. Effects of the prototype compound on rat carotid blood pressure and heart rate when administered intravenously (bolus injection, Panel a and b). Lidocaine's effects was included for comparison (Panel c).

From: "Wang, Desuo" <Wang@cop.sc.edu>
To: "Mike Muthig" <Muthigm@gwm.sc.edu>
Date: 4/24/03 5:20PM
Subject: RE: Wang, 341 Provisional patent application

Mike,
Here is the update of 341 summary. The research is still going on. So there will be more data in the futrue.
Thanks.

Desuo

—Original Message—
From: Mike Muthig [mailto:Muthigm@gwm.sc.edu]
Sent: Tuesday, April 22, 2003 2:00 PM
To: Wang, Desuo
Subject: Wang, 341 Provisional patent application

Thank you for meeting this morning to review the provisional patent application, which expires on May 31, 2003. As discussed, given the absences of a publication, new information that has been developed, and limited interest resulting from marketing efforts, the provisional patent application will be allowed to expire.

A new provisional patent application will be prepared that will include the following information:

Information included in the original application;
Information that was developed since the original submittal;
Information to be presented in your students defense;
Any other information that you would like to have included at this time.

Please forward any information that you would like to have included in the new application. I would like to submit the application prior to your students defense. I need at least 24 hours to prepare the application for submittal.

Please feel free to call if you have questions or comments.

Regards,

Micheal

****

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Novel Synthetic Antihypertensive and Antiasthmatic cAMP-specific Phosphodiesterase Inhibitors

General: the compounds are chemically synthesized and have a high yield. They are fairly soluble in acidic solution (~30 mg/ml, pH=3), light and heat stable, bioavailable *via* oral gavage, with a reasonable partition coefficient (i.e., with a calculated Logp value of 1.79), relatively low toxicity, reliable efficacy, and acceptable potency.

Preliminary Experimental Data

A. Effect on Blood Pressure

1. In anesthetized rats (SD, SHR, and Zuker *(fa/fa)*), administration of the compounds, *via* oral gavage (10 mg/kg.body weight), intravenous bolus injection (1 mg/kg.body weight), or continuous intravenous infusion (at a rate of 30 μg/kg.min for 10 min), significantly lowered the systolic and diastolic blood pressure measured from carotid artery.

2. In awake animals (SD, SHR, and Zuker *(fa/fa)* rats), administration of the compounds, *via* oral gavage (20 mg/kg.body weight) or intraperitoneal injection (1 mg/kg.body weight), also significantly lowered blood pressure measured with non-invasive tail-cuff method.

3. In anesthetized dogs, intravenous bolus injection of the prototypic compound at a dose of 0.25 mg/kg.bw produced a 4 mmHg decrease in blood pressure. Higher doses produced greater decrease in blood pressure.

	HR	BP Max	BP Min	BP Mean	LVSP	LVEDP
Control	119.8	99.8	46.6	67.2	78.5	8.7
Bolus 1 mg/kg.bw	118.2	90.5	39.8	58.4	71.8	9.0
Bolus 2 mg/kg.bw	109.7	76.8	33.7	48.0	63.1	9.2

HR, heart rate; BP, blood pressure; BP Max, maximum systolic pressure; BP Min, minimum diastolic pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure.

B. Effects on Heart Rate and Cardiac Contractility

1. *in vivo* studies in anesthetized or awake rats, the compounds did not produce obvious negative chronotropic effect when administered *via* oral gavage or intraperitoneal injection or intravenous infusion. The heart rate was markedly decreased when a bolus injection was applied to anesthetized rats.

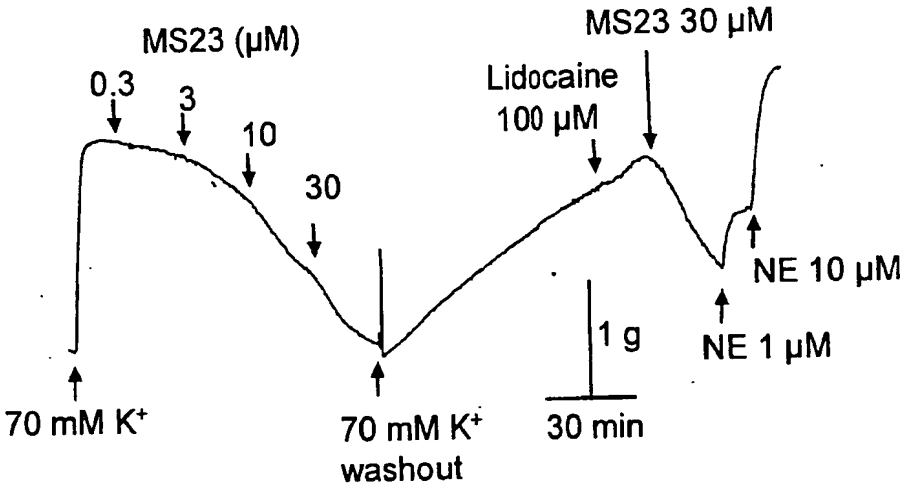
2. In Langendorff-perfused rat hearts, the compounds (30 μM) did not affect the heart beating rate and left ventricular contractility measured as left ventricular developing pressure (LVDP) using balloon method.

3. The effects of the compounds on atrium beating rate and contraction were also studied using tissue bath method. After the beating of the right atrium of

guinea pig was initiated and maintained at a constant rate by β-adrenergic agonist isoproterenol (200-500 nM), the compounds (30 μM) did not affect the positive chronotropic and inotropic responses of the atrium to isoproterenol stimulation. This is different from other phosphodiesterase inhibitors, such as 10 μM milrinone or 10 μM rolipram that markedly enhanced the positive chronotropic and inotropic effects of isoproterenol.

C. Relaxation Effects on the Blood Vessel Rings

1. *In vitro* experiments, the compounds fully relaxed high K^+-contracted (35, 70, or 140 mM) aorta rings of rats and guinea pigs. The relaxation was also effective when tested in intra-renal and coronary arterial rings (1~2 mm) as well as pig main kidney arterial and venous rings. The vasodilatation responses were endothelium independent, reversible upon washout, and lack of desensitization. The effects were not affected by elevating extracellular K^+ concentration from 70 mM to 140 mM. The efficacy and potency were similar in arterial and venous vessels, with a K_d value of approximate 15 μM.



MS23 relaxes high K^+ caused-contraction of porcine kidney artery ring.

2. The compounds also relaxed norepinephrine or phenylephrine-caused vessel constriction.

3. The relaxation action on 70 mM K^+- or norepinephrine-induced contraction was not affected in the presence of indomethacin (cyclooxygenase inhibitor), U73122 (PLC inhibitor), BQ-123 (ET_A antagonist), BQ-788 (ET_B antagonist), or CGS 15943 (A_1 and A_2 antagonist).

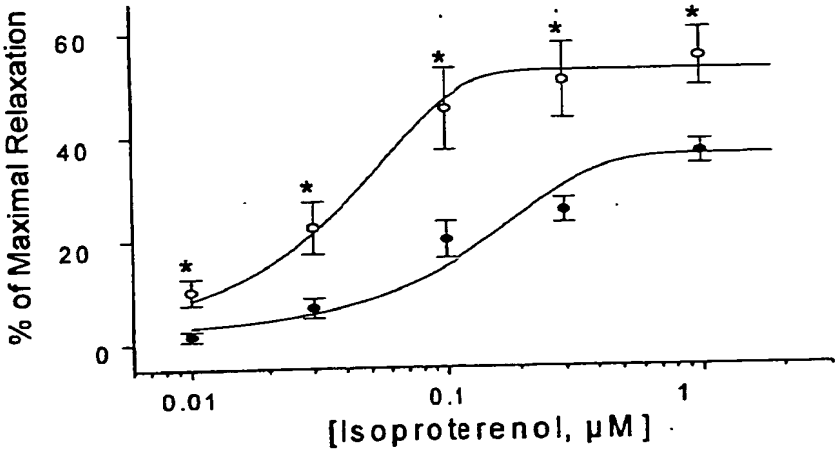
D. Relaxation Effects on Airway Smooth Muscles

1. The compounds fully relaxed high K^+-caused contraction of rat tracheal and bronchial rings (K_d=10 μM). The action was epithelial-independent. And the relaxation effect was not affected in the presence of the listed antagonists or inhibitors:

1) histamine H_2 receptor antagonist ranitidine (10-100 μM),
2) adenosine A_1 and A_2 receptor antagonist CGS 15943 (10 nM),
3) non-selective β-adrenergic receptor antagonist propranolol (1-5 μM) and selective β_2 and β_3 antagonists ICI 118511 (10 μM), SR59230A (5-10 μM), respectively, or α_1-adrenergic receptor antagonist prazosin (1 μM).
4) phospholipase C (PLC) inhibitor U73122 (3-10 μM),
5) cyclooxygenase (COX-1) inhibitor indomethacin (1-10 μM),
6) intracellular Ca^{2+}-release inhibitor ryanodine (10 μM),
7) adenylyl cyclase inhibitor 2'5'-dideoxyadenosine (30 μM),
8) nitric oxide synthase inhibitor Nitro-L-Arginine Methyl Ester (L-NAME, 100 μM),
9) non-selective PKC inhibitor staurosporin (1 μM),
10) T-type Ca^{2+} channel blocker Ni^{2+} (0.1-1 mM),
11) large-conductance (BK) Ca^{2+}-activated K^+ channel blocker TEA^+ (2 mM).

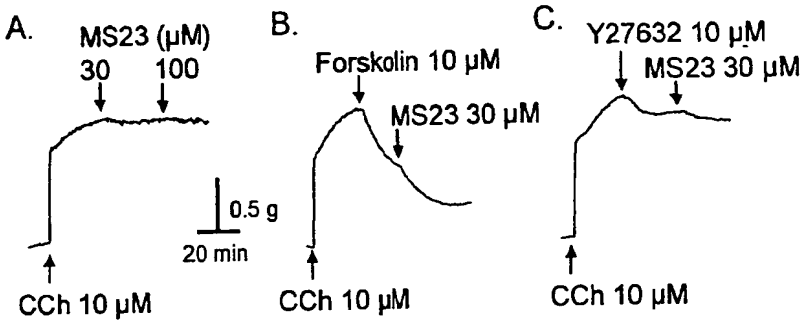
2. The compounds also relaxed histamine (100 μM)-caused contraction of guinea pig trachea and bronchi.

3. Although the compounds do not relaxed the maximal contraction induced by carbachol (10 μM) (only to ~10% of the maximal relaxation of atropine) in guinea pig airway smooth muscles, they markedly enhanced isoproterenol produced relaxation. The compounds caused a leftward displacement by at least one log unit of the concentration-response curve of isoproterenol-produced relaxation in airway rings contracted by carbachol (10 μM) and the relaxing efficacy increased from 30% to 45% of the maximal relaxation of atropine.



Concentration- response curve of isoproterenol-induced relaxation of rat bronchi rings contracted with 10μM carbachol in the absence and presence of 10μM MS23 (a concentration itself does not have relaxation effect on 10 μM CCh-caused contraction). MS23 increases the potency and efficacy of isoproterenol induced bronchodilation. Open circle: ISO+MS23; closed dot: ISO alone.

4. Pre-relaxation of 10 μM carbachol-caused airway contraction with forskolin (10 μM) significantly potentiated the compounds' relaxation effect. However, relaxation of carbachol-caused contraction by ROCK inhibitor, Y27632 (10 μM, selectively inhibiting the rho-associated coiled coil-forming protein kinase-ROCK) did not potentiate the compounds' action.



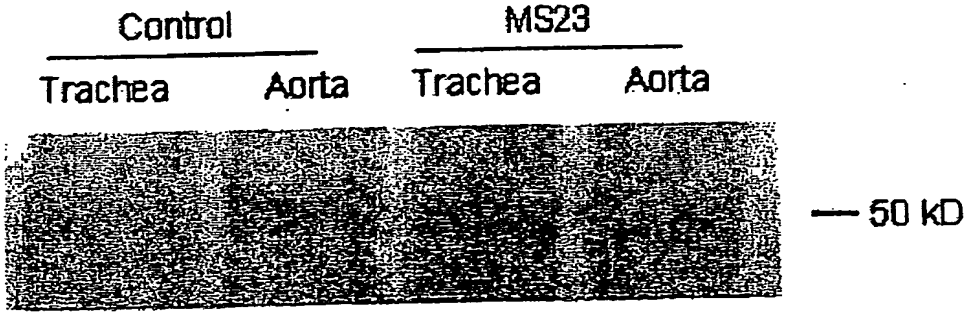
A. Carbachol-caused contraction of rat bronchial rings can not be relaxed by MS23 alone. B. In the presence of Forskolin, MS23 relaxes carbachol-caused contration. C. Y27632 Does not potentiate MS23 's action. (CCH: carbachol; Forskolin, adenylyl cyclase activator. Y27632, ROCK

5. In β-escin (100 μM) permeated tracheal smooth muscles, when free Ca^{2+} was buffered to 50 μM with BAPTA and ATP, the compounds relaxed calmodulin (10,000 units)-induced contraction.

6. In comparison to isoproterenol (1-3 μM) or calcium channel blocker (combination of 2 μM nifedippine and 5μM verapamil), the prototypic compound (30 μM) significantly attenuated the efficacy of Ca^{2+}-induced contraction, when rat airway rings were depolarized with 70 mM K^+.

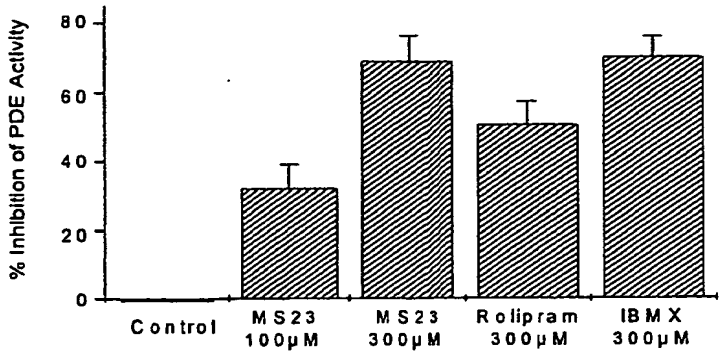
E. Vasodilator-stimulated Phosphoprotein (VASP) Assay

Vasodilator-stimulated phosphoprotein (VASP) is an actin filaments-associated protein that serves as a substrate for cAMP and cGMP-dependent protein kinases. Western blot carried out with phosphorylation-specific antibodies for VASP demonstrated that the 50 kD phosphorylated form of VASP was markedly increased in rat tracheal smooth muscle by the prototypic compound treatment for 30 min at a concentration of 100 μM (a concentration that can fully relax 70 mM K^+-caused contraction).



F. Phosphodiesterase (PDE) Assay

The prototypic compound concentration-dependently inhibited the total activity of cAMP phosphodiesterases (PDEs) contained in the protein extract from brain tissue and airway smooth muscles.



Inhibition of phosphodiesterase activity by MS23 measured as the ratio of conversion of cAMP to 5'-AMP using ^{3}H-cAMP radioisotope scintillation.

G. Other Results

1. The compounds (100 μM) failed to stimulate cAMP production in rat adipose cells.

2. The compounds (100 μM) did not relax high K^+-caused contraction of pig ureteral or guinea pig intestine (ileum and duodenum) rings that could be fully relaxed by 10 μM salbutamol (a selective β_2-adrenergic receptor agonist) or combination of verapamil (5 μM) and nifedipine (2 μM) or milrinone (a PDE3-selective inhibitor).

3. Patch-clamp recordings showed that the compound did not cause changes in action potential duration and amplitude in both rat and guinea pig ventricular myocytes. The compounds also did not change resting membrane potential. In contrast, PDE inhibitor milrinone (10 μM) significantly prolonged action potential duration of guinea pig ventricular myocytes.

4. There were no observed adverse effects in 6 Zucker *(fa/fa)* rats that continuously received a single daily dose (40-60 mg/kg.body weight) of the prototypic compound *via* oral gavage for three weeks. The compounds were non-emetic in the rats at a dose range of 20-80 mg/kg.bw (oral gavage).

Potential Areas of Use

1. Treatment of cardiovascular conditions (including systemic hypertension, angina pectoris, hypertension-related stoke and heart failure).
2. Treatment of asthma: as a monotherapy or as an adjunct therapy to enhance the therapeutic effect of the existing β-adrenergic agonists in asthma treatment, and reduce the dose, therefore, the adverse effect of the current used agents.

Main Advantages

1. Fast action (response occurs within 30 min after oral gavage, <3 min after intraperatoneally, immediate after intravenously).
2. Less adverse effects (less action on heart rate and myocardium contraction; mild action; no hypotensive-caused reflex reaction, no emetic reaction) and low toxicity.
3. No desensitization.
4. Effects are reversible and reproducible.
5. Relatively selective in action on airway and vascular smooth muscles.
6. Similar potency and efficacy in arteries and veins.
7. Significantly improvement in solubility
8. Reduced therapy cost.

Chemistry

1. The structure and purity (organic) have been verified by NMR (H^+ and C^{13}) measurements (>99.9% purity).
2. The structure and molecular weight have been confirmed using high resolution mass spectrometry (<0.02% of discrepancy from calculated molecular weight).

The preliminary data indicates that the compounds may selectively relax airway and vascular smooth muscles by inhibiting a subtype 4 cAMP-specific phosphodiesterase.

The antihypertensive activity of a new vasodilator MS23

Background: 2-[2-(N-ethyl-N-n-propyl) amino] propionamido-3-carbamyl-4-methyl-5-(methylthioethyl) pyrrole (MS23) is a new synthetic vasodilator, which selectively inhibits the enzymatic activity of cyclic AMP specific phosphodiesterase to cause the relaxation of vascular and air way smooth muscles. This study is to evaluate the antihypertensive effect and the potential action of MS23 on heart rate and cardiac contraction using *in vivo* and ex *vivo* models. Method and results: 1. In anesthetized SHR and SD rats, MS23 intravenous bolus injection at a dose of 0.5 mg/kg body-weight resulted in a decrease in the mean blood pressure (measured at carotid artery) by -15.6 mmHg (-16.1 and -15.4 mmHg for systolic and diastolic pressure, respectively), and by -45.8 mmHg (-45.2 and -46.1 mmHg for systolic and diastolic pressure, respectively) at a dose of 4 mg/kg body weight. The decrease in BP occurred immediately after MS23 administration and lasted for 3 to 6 min dependent on the dose. In anesthetized dogs, intravenous bolus injection of MS23 at a dose of 0.25 mg/kg produced a 4 mmHg decrease in blood pressure and higher dose produced greater decrease in blood pressure. 2. Continuous intravenous infusion of MS23 at a speed of 1.85 μg/min produced a steady-state reduction of the MBP by -28.3 mmHg (systolic –27.2 and diastolic –28.9 mmHg). 3. In awake SHR and SD rats, administration of MS23 via oral gavage (40 mg/kg body-weight) or intraperitoneal injection (20 mg/kg body-weight) also significantly lowered MBP (measured with non-invasive tail-cuff method) by -18.4 mmHg (systolic -20.3 and diastolic -17.4 mmHg) and -35.2 mmHg (systolic -35.4 diastolic -35.1 mmHg), respectively. 4. In anesthetized animals, high dose of MS23 (>2 mg/kg body-weight) produced a negative chronotropic effect when via intravenously administrated (bolus). The average decrease in heart rate was ~15 beat/min. The negative chronotropic effect was not observed when MS23 was administrated via oral gavage (40 mg/kg.bw), intraperitoneal injection (20 mg/kg.bw) or intravenous infusion (30 μg/kg.bw). 5. In Langendoff-perfused rat hearts, MS23 (30 μM) did not affect the heart rate and left ventricular contractility measured as left ventricular developing pressure (LVDP) via a balloon inserted in the left ventricle. Conclusion: MS23 potently and non-preferably reduces both systolic and diastolic blood pressure with negligible action on heart rate and cardiac contractility.

The Electrophysiological Effects of a Novel cAMP Specific Phosphodiesterase Inhibitor MS23 in Isolated Guinea Pig Cardiomyocytes

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The effects of a novel synthesized cAMP specific phosphodiesterase inhibitor MS23 on action potential parameters were examined and compared with those of milrinone (10 μmol/l, a PDE III inhibitor) and rolipram (30 μmol/l, a selective PDE IV inhibitor) in isolated guinea-pig atrial and ventricular myocytes. Action potentials (AP) were recorded under nystatin-perforated whole-cell patch-clamp configuration at a temperature of 35±0.5°C. MS23 at a concentration of 30 μM significantly shortened APD_{90} (action potential duration measured at 90% of repolarization) from 304.9±24.0 msec to 254.9±28.4 msec ($P<0.05$, n=8) in ventricular cells but had no effect on APD in atrial cells. MS23 did not affect the amplitude, resting membrane potential, and the rate of depolarization. MS23 did not prolong APD at concentrations range from 1 to 100 μM. In comparison, rolipram (30 μM) also shortened APD_{90}) from 318.3±8.9 msec to 274.6±9.8 msec ($P<0.05$, n=3) in ventricular cells but consistently showed a small but notable delay of phase 1 and plateau phase but acceleration of phase 3 repolarization. In contrast, milrinone (10 μM) elongated ventricular APD_{90} from 287.9±10.3 msec to 327.8±17.4 msec ($P<0.05$, n=4). Nevertheless, after treatment of ventricular cells with either rolipram or MS23, milrinone (10 μM) markedly shortened APD_{90} recorded from the same cells. In atrial cells, milrinone caused a greater delay of phase 1 and plateau phase but acceleration of phase 3 repolarization (APD_{90} shortened from 153.9± 12.1 msec to 135.5±12.3 msec, $P<0.05$, n=4). The results indicate that MS23 may have a different PDE inhibition spectrum from rolipram and milrinone. The PDE sensitive to MS23 may exclusively expressed in ventricular myocardium.

Investigation of MS23 induced smooth muscle relaxation

We investigated the smooth muscle relaxation action of 2-[2-(N-ethyl-N-n-propyl) amino] propionamido-3-carbamyl-4-methyl-5-(methylthioethyl) pyrrole (MS23), a novel synthetic antihypertensive and antiasmatic reagent . MS23 relaxed 70mM KCl contracted blood vessel and airway smooth muscle rings from rat and guinea pig, in a concentration-dependent manner, with an EC_{50} value of approximate 10 μM. The effects were endothelium independent . MS23 also relaxed norepinephrine/phenylephrine caused vessel contraction and histamine (100μM) contracted guinea pig trachea and bronchi. MS23 showed different tissue specificity against L-type Ca2+ channel blockers nifedipine (2μM) and Verapamil (5μM). In carbachol (10μM) contracted guinea pig smooth muscle rings (trachea and bronchi), pretreatment of MS23 (10μM, at which MS23 relaxed less than 10% of the maximal relaxation by atropine) potentiated the relaxation incuced by isoproterenol (0.01-10μM) significantly. Moreover, forskolin (10μM, adenylyl cyclase activator) potentiated MS23 (30μM) induced relaxation significantly (5 folds), which indicated the interference of MS23 in the signaling of cyclic AMP induced smooth muscle relaxation. The enzymatic activity measurement of phosphodiesterases (PDE, sample from guinea pig brain protein extract) which converted cAMP to 5'-AMP showed that MS23 inhibited PDE activity concentration dependently. Conclusion: MS23 relaxed smooth muscle tissue by the inhibition of cyclic AMP specific phosphodiesterase.

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